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# Advanced Research Center for Beam Science - Structural Molecular Biology -

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## Scope of Research

The research activities in this laboratory are performed for X-ray structural analyses of biological macromolecules and the investigation of the electronic state in materials as follows: The main subjects of the biomacromolecular crystallography are crystallographic studies on the reaction mechanism of enzymes, the relationship between the multiform conformation and the functional variety of proteins, and the mechanism of thermostabilization of proteins. In the investigation of the chemical state in materials, the characteristics of the chemical bonding in the atom and molecules are investigated in detail using a newly developed X-ray spectrometer with a high-resolution in order to elucidate the property of materials. The theoretical analysis of the electronic states with DV- $X\alpha$  and WIEN2k, and the development of new typed X-ray spectrometer with ultra high-resolution have also been carried out.

## Research Activities (Year 2008)

### Publications

Fujii T, Goda Y, Yoshida M, Oikawa T, Hata Y: Crystal-  
lization and Preliminary X-ray Diffraction Studies of  
Maleylacetate Reductase from *Rhizobium* sp. Strain  
MTP-10005, *Acta Cryst.*, **F64**, 737-739 (2008).

Fujii T, Oikawa T, Muraoka I, Soda K, Hata Y: Crystal  
Structure of Tetrameric Malate Dehydrogenase from Antarctic  
Psychrophile, *Acta Cryst.*, **A64**, C255-256 (2008).

### Presentation

Crystal Structure of Tetrameric Malate Dehydrogenase  
from Antarctic Psychrophile, Fujii T, Oikawa T, Muraoka  
I, Soda K, Hata Y, XXI Congress and General Assembly  
of the International Union of Crystallography, 29 August  
2008, Osaka, Japan.

## X-Ray Diffraction Studies of GraC Involved in Resorcinol Catabolism of *Rhizobium*

*Rhizobium* is a genus of tubercle-forming bacteria. It grows in the root of a plant in symbiosis with other bacteria to fix nitrogen from the air. Although much attention has been paid to the *Rhizobium* genes and gene products, there is still little information available on the molecular structure, function, and detailed properties of the enzymes involved in its metabolic pathways. In the course of a screening experiment, *Rhizobium* sp. strain MTP-10005 was isolated from natural river water. Enzymological studies showed that the *graD*, *graA*, *graB*, and *graC* genes of the bacterium encode the reductase (GraD) and oxidase (GraA) components of resorcinol hydroxylase, hydroxyquinol 1,2-dioxygenase (GraB), and maleylacetate reductase (GraC), respectively. In order to reveal their structures and functions, we have been performing X-ray structural studies of the enzymes.

Maleylacetate reductase (GraC) from *Rhizobium* sp. strain MTP-10005 catalyzes NADH- or NADPH-dependent reduction of maleylacetate to 3-oxoadipate. The polypeptide chain of the enzyme consists of 351 amino acid residues. The amino-acid sequence of the enzyme is homologous to those of maleylacetate reductases from *Ralstonia eutropha* JMP134, *Pseudomonas* sp. strain B13, and *Agrobacterium tumefaciens*. These homologous enzymes are known to be inhibited by thiol-blocking reagents such as *p*-chloromercuribenzoate and  $\text{Hg}^{2+}$ . The sequence homology suggests that this inhibition might be conserved in the present enzyme GraC.

Initial crystallization experiments were performed by the hanging-drop vapour-diffusion method using Crystal Screens I and II (CS I, II). The final conditions based on those of CS II#32 produced rhombohedron-shaped crystals with approximate dimensions of  $0.30 \times 0.20 \times 0.05$  mm at 293 K in 3 d using the sitting-drop vapour-diffusion method (Figure 1). Drops of 1  $\mu\text{l}$  protein solution at 8 mg  $\text{ml}^{-1}$  (in 50 mM Tris-HCl buffer, pH 8.0) and 1  $\mu\text{l}$  reservoir solution were equilibrated against 500

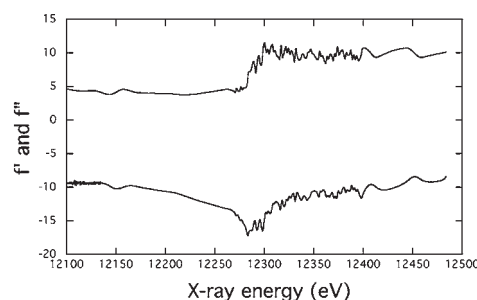


**Figure 1.** Crystal of maleylacetate reductase (GraC) from *Rhizobium* sp. strain MTP-10005. The dimensions of the crystal were approximately  $0.30 \times 0.20 \times 0.05$  mm.

$\mu\text{l}$  reservoir solution consisting of 1.4 M ammonium sulfate, 0.1 M sodium chloride, 2% (w/v) benzamidine HCl, and 0.1 M NaHEPES, pH 7.5. The drops were microseeded using the original crystals grown in CS II#32.

Diffraction experiments were performed at beamline BL6A, Photon Factory, Tsukuba, Japan. The native crystal with typical dimensions  $0.25 \times 0.20 \times 0.05$  mm was soaked into a cryoprotectant solution consisting of 1.4 M ammonium sulfate and 25% (v/v) glycerol for less than ten seconds, and flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected at a wavelength of 1.000 Å using a Quantum 210 CCD detector set to 166.2 mm in a crystal-to-detector distance. The data set was collected at 1.96 Å resolution and has 44,689 independent reflections with completeness of 99.5%.

To solve the phase problem, one kind of heavy-atom isomorphous derivative crystal was prepared by soaking the native crystal in the reservoir solution containing 0.025 mM ethylmercury thiosalicylate (EMTS) for 20 hours. Then, the multi-wavelength anomalous diffraction (MAD) method was applied for solution of the phase problem. Before data collection, XAFS experiments were carried out with this derivative crystal. The absorption spectrum obtained from this experiment was used to fix the wavelengths at four sites (Peak, Edge, Remote-H and Remote-L) around the X-ray absorption L-edge of  $\text{Hg}^{2+}$  (Figure 2). Besides, the spectrum was analyzed to determine the dispersion and absorption components ( $f'$  and  $f''$ ) of  $\text{Hg}^{2+}$  for the anomalous dispersion correction at their wavelengths (Table 1).



**Figure 2.** Anomalous scattering factors near the absorption L-edge of mercury from a derivative crystal.

**Table 1.** Wavelengths and anomalous scattering factors obtained from XAFS experiments

	Peak	Edge	Remote-H	Remote-L
Wavelength (Å)	1.00798	1.00940	0.99923	1.01352
$f'$	-14.56	-17.22	-8.54	-11.99
$f''$	11.49	6.50	10.09	3.92

The MAD data sets were collected at 3 Å resolution for the same derivative crystal by irradiation of X-rays with the above four wavelengths. Each of four data sets has about 12,800 independent reflections with completeness of over 99.5%. The structure analysis of GraC is underway in calculating phase angles and electron densities.